

Phytoremediation of Soil Trace Elements

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14.1 Introduction

Phytoremediation includes many uses of plants to achieve remediation of soil risks. In relation to soil trace elements (hereafter, metals), the focus is phytoextraction, phytovolatilization, and phytostabilization. *Phytoextraction* uses growing and harvest of plants which accumulate high quantities of metals in shoots, allowing their removal from a contaminated site. If the plant biomass can be used as an alternative ore with monetary value, this can be labeled *phytomining*. *Phytovolatilization* uses plants and soil microbes to transform soil elements (Se, Hg) into volatile forms which leave the soil ($(\text{CH}_3)_2\text{Se}$, Hg^0). *Phytostabilization* uses soil amendments which can cause formation of chemical forms of the metal with lower phytoavailability and bioavailability. The use of plants to prevent erosion and to support a sustainable ecosystem safe for wildlife is also part of phytostabilization. These approaches are considered 'green' technologies, utilizing low-cost agricultural practices rather than earth-moving equipment.

Much progress has been made in understanding phytoremediation processes in the last twenty years. Because phytoextraction effectiveness depends on the amount of metals accumulated in the harvestable biomass per year, rare plants which hyperaccumulate metals in their shoots are especially valuable [1]. The word 'hyperaccumulator' was coined in 1977 [2], although Jaffré and Schmid [3] earlier used the French word *hypernickelophore*, while 'phytoextraction' was coined in 1983 [4]. The earliest review of the use of hyperaccumulator plants for phytoextraction was by Baker and Brooks [5]. These authors [5] suggested that species which accumulated Zn or Mn at 10 000 mg kg⁻¹ dry weight (DW), Ni, Co, Pb or As at 1000 mg kg⁻¹ DW, and Cd at 100 mg kg⁻¹ DW could be called hyperaccumulator species. The levels noted are about 100 times higher than found in normal plants under most circumstances. Debate continues about what the hyperaccumulator concentration should be for each element, but the listed figures have received wide acceptance. Reeves [6] provided a fuller definition for Ni hyperaccumulators that should be applied to all elements and hyperaccumulator species: '... it is suggested that a hyperaccumulator of nickel be defined as "a plant in which a nickel concentration of at least 1000 mg kg⁻¹ has been recorded in the dry matter of any aboveground tissue in at least one specimen growing in its natural habitat".' The implication of this definition is that tests which use artificial soils, spiked soils, or nutrient solutions with single elements added do not reflect the natural environment of hyperaccumulator plants, or of multielement contaminated soils. Many claims of hyperaccumulation based on Cd-only spiked soils are senseless because geogenic soil Zn concentrations are usually at least 100 times that of Cd. Therefore Zn inhibition of Cd uptake will easily prevent practical Cd phytoextraction by the claimed species (for example, [7]). If crop plants have $\geq 25\%$ yield reduction at 500 mg Zn kg⁻¹, leaves will reach ≤ 5 mg Cd kg⁻¹ even if they could attain > 100 mg Cd kg⁻¹ in a Cd spiked soil or nutrient solution. Relevant tests must be done with field multielement contaminated soils.

For phytoextraction to be accepted for remediation of contaminated soils, the process must be cost effective. If no one will pay for remediation of a contaminated site to reduce potential risks to humans and the environment of soil metals, no actions will be taken. Thus the costs of growing a crop to conduct phytoremediation must recover the costs of growing and harvesting and use or disposal of the crop. Producing value in the crop can offset these costs and support soil remediation.

Several authors have developed economic models that illustrate these concepts. In the case of soil selenium (Se), Banuelos and Mayland [8] showed that biomass of a Se-rich phytoremediation crop (that also did some phytovolatilization of Se) could be mixed in livestock feeds and replace Se salts normally added to feeds. Considering the difficulty of adding 0.2–1.0 mg Se kg⁻¹ to feed, mixing ground Se-rich biomass would likely make better-mixed feeds than adding very small amount of Se salts. Analogously, Se-enriched vegetable crops might be marketed as phytonutrient-enhanced or naturally biofortified foods. Because of high sulfate levels in the same soils, crop Se is limited to levels which are safe in foods [9]. Robinson *et al.* [10] note the cost aspect for designing a phytotechnology to alleviate risk from boron in leachate from a treated wood waste pile, and discuss a model to estimate value of products and costs of phytoremediation.

Several groups have proposed growing willow as a biomass energy crop with an additional benefit of Cd removal if specific cultivars are utilized (for example, [11]). If enough Cd was removed from the soil, the ash would have to be placed in a landfill rather than recycled on forest land to return the nutrients removed in growing the crop (see

discussion below). In this cost model, unless farmers considered the reduced soil Cd as a value to their land, they would not accept the loss in higher-value vegetable crop production due to growing willow to remove Cd [11]. Thus, unless the sale of food crops required soil remediation according to regulations, farmers would not consider remediation. Although the European Union (EU), United States, Japan, and China have much land which contains Cd contamination, governments have not ordered remediation or paid for remediation; therefore little has been undertaken other than research and development of Cd phytoextraction technology.

Phytoextraction using hyperaccumulators could be a cost-effective method to mine Ni from soils [12], or to remediate Ni phytotoxic soils. Ni metal sells for \$15 kg⁻¹ at this writing; it had reached more than \$50 kg⁻¹ in 2007. Ash from incineration of *Alyssum murale* biomass containing about 20% Ni is easily used as an ore in electric furnace refining of Ni. Plant ash is free of interfering factors (such as Fe and Mn oxides and silicates) present in lateritic Ni ores that require expensive processing to release Ni. An effective crop of 20 t ha⁻¹ dry biomass containing 2.0% Ni yields 400 kg Ni ha⁻¹, and offers more profit than most agronomic crops, especially considering the infertile serpentine soils which are Ni phytomining substrates. Alternatively, the hyperaccumulator biomass can be used as an organic Ni fertilizer [13].

However, other elements in the plant biomass or biomass ash are seldom valuable enough to cover the costs of growing the crop. For example, phytoextracted Cd has negligible value; not even the sum of Cd and Zn has enough value to offset the costs of crop production and harvest. Similarly, crop Pb or As is unmarketable, hence plant biomass must be placed in a landfill – generating another cost as opposed to offsetting cost. Chaney *et al.* [12] discuss the disposal of plant biomass from phytoextraction. In contrast with some claims [14], phytoextraction biomass disposal is not a difficult problem, only a disposal cost rather than a source of profit.

Considerable research has been conducted on phytoextraction of soil metals, and several authors have noted problems encountered in development of commercial technologies. For example, Robinson *et al.* [15] list these potential limitations in using phytoextraction to remediate contaminated sites: (i) long period required for cleanup; (ii) limited number of target metals which can be phytoextracted; (iii) limited depth that can be accessed by roots; (iv) difficulty in producing a high biomass crop of the desired species; (v) potential of plant metals to enter environmental food chains; and (vi) inevitable leaching of metals if chelators are added to induce phytoextraction.

Ernst [16,17] has rightly stressed the ‘hype’ of hyperaccumulator use in phytoextraction. Many who have conducted research on hyperaccumulators did not appreciate the complex soil chemistry of trace elements, and the difficulty of moving polyvalent cations through plant membranes. It is nearly impossible to achieve useful phytoextraction of Pb, Cr, Cu, and some other important trace elements because they are too insoluble in soils or retained in plant roots. Adding chelating agents to ‘induce’ phytoextraction was never a good idea; it causes leaching of metals to groundwater, and is extremely expensive. Ernst [18] initially stressed the poor yield and low metal concentration in biomass harvested from sites where *Thlaspi caerulescens* occurred along with metal tolerant grasses. This criticism is valid in that the full yield of *T. caerulescens* is not high, but a reduced yield due to competition/shading from other species can be controlled by selective herbicides. Ernst also notes the potential difficulty of public acceptance of transgenic

metal hyperaccumulator crops made from crop species. We should listen carefully to Dr. Ernst's comments because of his extensive experience with metallophytes across Europe [19]. Most of the early literature on *Thlaspi caerulescens* came from ecological and physiological studies by Ernst and cooperators. And Russian biogeochemists/botanical prospectors [20] reported Ni hyperaccumulators before the surge of research since 1977. Ernst, Brooks and Baker are the 'fathers of phytoextraction' who helped spread the idea which has led to development of important knowledge and technology.

On the other hand, Ernst [16] failed to consider the use of agronomic management practices to maximize yields of the hyperaccumulator crops. Appropriate use of herbicides and tillage, and optimal use of fertilizers and soil amendments to adjust pH and improve productivity can give useful harvestable yields of the wild plants. Grasses overwhelm the biomass of *T. caerulescens* if not controlled by herbicide, and may strongly reduce annual phytoextraction quantity. And any plant breeder would at least suggest that, given time, they could improve the harvestable yields of even rosette plants such as *T. caerulescens*.

Biotechnology is widely believed to offer the ability to combine critical activities of hyperaccumulator plants into plants with high harvestable biomass yields, which are easier to produce as an agricultural crop. How many proteins are required to transform a normal plant into a *T. caerulescens*, *Alyssum murale* or *Pteris vitatta* equivalent hyperaccumulator is simply not known, but recent research still supports the view that translocation from root to shoot, and storage in leaf cell vacuoles are the key functions needed [21]; selectivity of the root uptake transporter may also contribute, and may increase metal tolerance. But the 'hypertolerance' noted for hyperaccumulators is mostly dependent on effective storage in leaf cell vacuoles. Only continuing research and development will determine whether cost-effective bioengineered phytoextraction crops will become available for commercial use. Improved cultivars of natural hyperaccumulators have already been used in the field [22]. And it may take production of sterile hybrids of phytoextraction plants to allay fears of transferring metals into environmental food chains, especially for transgenics.

Although this review describes methods for phytoextraction which appear to be cost effective and ready to apply, one should always consider phytostabilization rather than phytoextraction for many elements. For Ni and Zn phytotoxic soils, a ready immediate remediation is available via making the soil calcareous and adding appropriate fertilizers to maintain soil fertility [23–28]. Only if the value of Ni or Zn in the plant biomass can support phytomining would phytoextraction be a better alternative than phytostabilization.

Some have suggested that yields of all hyperaccumulators are too low to give useful phytoextraction of soil metals [29]. This claim is contradicted by the effective phytomining of Ni by *Alyssum* species, which can provide more net income than growing normal crop plants on fertile soils [12]. Useful phytoextraction cannot be achieved by crop plants except in the case of Se [8] and that opportunity still requires development. Plants for phytoextraction must be highly metal tolerant, and accumulate high concentrations of the target elements in harvestable shoots so that the annual removal of metal from the site is economic.

Because of the unusual potential of these 'green' technologies to achieve soil remediation, much research has been conducted and reported. Substantial progress has been made even on the genetics of metal hyperaccumulator species, and the biology and biochemistry of phytoextraction. At the same time, much research has used addition of chelating agents to conduct 'induced phytoextraction,' but the added chelators cause leaching of target or

other metals to groundwater. And many papers have reported studies of plant species with no promise for practical phytoextraction whatsoever because the researchers did not understand the practical side of soil contamination and hyperaccumulation. For example, species such as *Brassica juncea* never had promise for practical phytoextraction; it is not a metal tolerant species, and does not accumulate Pb or other elements from soils without addition of chelators. Even for Se phytoextraction, *B. juncea* has little value because it does not accumulate Se in the presence of high levels of sulfate normally found in Se contaminated or mineralized soils as do the Se hyperaccumulators.

14.2 The Nature of Soil Contamination where Phytoextraction may be Applied

Remediation of contaminated soils is conducted in response to government decisions about land use, or to provision of funding for remediation. Farmers may desire Cd removal from soils with mine or smelter contamination, where high Cd-phosphates were applied for decades, or high Cd biosolids were applied before regulations were developed, but the decision to proceed will be based on the economics of their farm operation. As noted below, the main impediment to development of phytoextraction technology is the failure of government to require and fund soil remediation. Usually the contamination must be severe enough to markedly limit crop selection, or to produce barren soils or unsafe food before government acts. The situation is complex because acidic soil pH increases soil metal phytoavailability and simple application of limestone may reduce metal phytotoxicity and metal uptake by crop plants and restore crop production freedom. When severe contamination occurs from mine wastes or smelter emissions, soils are often acidic, highly contaminated, and simple limestone application does not restore crop cover. In the United States, the Superfund program deals with such contaminated soils if humans or aquatic ecosystems are threatened by the soil contaminants, but simple soil ecosystem disruption is not usually enough to trigger Superfund status and action by government.

Soil metals must be present in chemical forms/solubilities which plants can absorb and translocate to shoots or the phytoextraction option is null. That is why Cr(III), Pb, Sn, and many other elements have such poor absorption, and Pb is also trapped within the fibrous root system as insoluble lead phosphate. Plants can only absorb metals from the volume of soils that root explore. High density of plant roots is generally limited to the surface 0–15 or 0–30 cm of soil depth, so metals in deeper soil layers are not likely to be phytoextracted effectively. Tap roots that obtain water from deeper soil depths may not contribute to metal accumulation. Deep-rooted hyperaccumulators, and trees grown with roots at depth within 10-m deep wells [30] to provide access to deeper soils can achieve removal of groundwater and contaminants from deeper soil than the surface 0–30 cm.

Tillage of contaminated soils may cause dispersal of the contamination by rainfall, especially on slopes. Phytoextraction can be applied only where the soil can be tilled, or competing plants controlled by selective herbicides, and the shoot biomass harvested mechanically. Fortunately, methods have been developed for surface application of amendments (organic matter plus limestone and fertilizers) on highly sloping contaminated soils, which can achieve effective phytostabilization of these soils [27].

14.3 Need for Metal-Tolerant Hyperaccumulators for Practical Phytoextraction

Soils which need phytoremediation for metals are often barren or have cover with only a few species of metal-tolerant plants [31]. Although some authors propose to grow crop plants because of potentially high yields of shoot biomass, if the species cannot tolerate the soil metals, one must modify the soil or choose another species for cover. The exceptional tolerance of shoot metals by hyperaccumulator plants has brought attention to these unusual species since at least 1960 [32]. Brooks [33] reviewed hyperaccumulator plants and covered many aspects of these unusual species. Table 14.1 lists identified element hyperaccumulator species with more than 1% of metal in shoots as sampled in the field on contaminated or mineralized soils.

In introducing the concept of phytoextraction, Chaney [4] provided an example of growing corn (*Zea mays* L.) or *Alyssum murale* on a Ni-mineralized or contaminated site (Table 14.2). Even at 50% yield reduction with 100 mg Ni kg⁻¹ dry shoots, the high-yielding corn shoots contained only 1 kg Ni ha⁻¹. It should be clear from this example that crop plants cannot absorb high enough amounts of Ni to support phytoextraction. Annual removal of Ni is too small for crop plants even when they are suffering considerable yield reduction. However, even moderate yielding species such as *Alyssum murale* or *A. bertolonii* can accumulate 1–2% Ni [22,34,35], and improved agronomic management (fertilizers, herbicides, etc.) and cultivars produced by plant breeding can accumulate more than 3% Ni in dry shoots [12,22].

Although crop plants can accumulate higher levels of Zn than Ni before yield is substantially reduced, claims that crop plants may be useful for phytoextraction [36]

Table 14.1 Example plant species which hyperaccumulate elements to over 1% of their shoot dry matter; usually to at least 100-fold levels tolerated by crop species

Element	Plant species	Maximum metal concentration (mg kg ⁻¹ DW)	Location collected	Reference
Zn	<i>Thlaspi caerulescens</i> ^a	39 600	Germany	275
Cd	<i>Thlaspi caerulescens</i>	2908	France	77
Cu [†]	<i>Aeolanthus biformifolius</i>	13 700	Zaire	149
Ni	<i>Phyllanthus serpentinus</i>	38 100	New Caledonia	276
Co ^b	<i>Haumaniastrum robertii</i>	10 200	Zaire	148
Se	<i>Astragalus racemosus</i>	14 900	Wyoming, USA	277
Mn	<i>Alyxia rubricaulis</i>	11 500	New Caledonia	278
As	<i>Pteris vittata</i>	22 300	Florida, USA	210
Tl	<i>Biscutella laevigata</i>	15 200	France	177

^aIngrouille and Smirnoff [279] summarize consideration of names for *Thlaspi* species; many species and subspecies were named by collectors over many years [275,280,281].

^bAlthough Cu and Co hyperaccumulation were confirmed in field collected samples, similar concentrations have not been attained in controlled studies.

Reproduced from Chaney, R.L.; Angle, J.S.; Broadhurst, C.L.; Peters, C.A.; Tappero, R.V.; Sparks, D.L., Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies; J. Environ. Qual. 2007, 36, 1429–1443 [12].

Table 14.2 Estimated Ni phytoextraction by corn (*Zea mays* L.) vs. *Alyssum murale* grown as a phytomining crop (adapted from [4,12]); assume control soil contains 25 mg Ni kg^{-1} , and the Ni-rich soil contains $2500 \text{ mg Ni kg}^{-1} = 10\,000 \text{ kg Ni (ha } 30 \text{ cm)}^{-1}$; assume soil Ni is sufficiently phytoavailable that corn has 50% yield reduction compared to corn grown on similar soil without Ni mineralization. Research has shown that unimproved *Alyssum murale* can easily yield 10 Mg ha^{-1} with fertilizers, and selected cultivars can exceed 20 Mg ha^{-1} with appropriate soil and crop management on serpentine soils [42]. Most crop plant species suffer 25% yield reduction when the shoots contain $100 \text{ mg Ni kg}^{-1}$ dry weight [24]. Ni concentration in ash is limited by formation of NiCO_3 with only 49% Ni

Species	Soil	Yield (dry Mg ha^{-1})	Ni in the crop			Ash-Ni (%)
			(mg kg^{-1})	(kg ha^{-1})	(% of soil)	
Corn	Control	20	1	0.02	0.01	0.002
Corn (50% YD)	Ni-rich	10	100	1.	0.01	0.20
Wild <i>Alyssum murale</i>	Ni-rich	10	20 000	200.	2.0	20–25
<i>Alyssum murale</i> cultivar	Ni-rich	20	25 000	500	5.0	25–30

Reproduced from Chaney, R.L.; Angle, J.S.; Broadhurst, C.L.; Peters, C.A.; Tappero, R.V.; Sparks, D.L., Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies; J. Environ. Qual. 2007, 36, 1429–1443 [12].

ignore the evidence of Zn phytotoxicity at $<1000 \text{ mg Zn kg}^{-1}$. As will be discussed below regarding phytoextraction of soil Cd, most soils have 200-fold higher concentration of Zn than Cd, so if the plant does not tolerate very high Zn levels, it cannot survive to phytoextract soil Cd.

Some researchers have predicted that phytochelatins would be important in metal tolerance and accumulation in plants, but research has shown these compounds have no identifiable role in Zn–Cd-hyperaccumulator plants [37,38]. Bioengineering of plants to express several enzymes which may increase the biosynthesis of phytochelatins caused only a small increase in plant metal tolerance or accumulation, far below those of natural hyperaccumulators. Clearly, phytochelatins are not a part of the hyperaccumulator phenotype.

14.4 Phytoremediation Strategies: Applications and Limitations

14.4.1 Phytomining Soil Nickel

Nickel (Ni) provides the best example for using natural hyperaccumulator plants to phytoextract soil Ni for profit [12]. The Ni phytomining technology [39] was developed by selecting for development promising high-yielding species with strong Ni

hyperaccumulation for testing under controlled conditions. Seeds of diverse genotypes were collected where native plants grew in southern Europe. The composition of field-collected specimens is influenced by many factors which vary between sites and collectors; thus, only a field comparison of species or genotypes of a species can show the true potential of any species or genotype.

A growth test was conducted on serpentine soils in southwestern Oregon, USA. Greenhouse and growth chamber studies showed the seeding depth required for obtaining effective germination, and fertilizers and pH adjustment necessary to get maximum yield and Ni accumulation on infertile serpentine soils [22]. Greenhouse testing showed which herbicides did not harm *Alyssum* while controlling species which commonly grow on these serpentine soils (mostly grasses). Testing also showed that *Alyssum murale* and *Alyssum corsicum* were self-incompatible, so breeding to improve cultivars required use of recurrent selection rather than simpler methods. To minimize issues of variation in the field, reference genotypes were grown in each planting block with six other entries, and covariance correction for the reference genotype Ni concentration was used in genotype comparisons. Substantial genetic variation in shoot Ni concentration was found in both species evaluated (Figure 14.1).

Among other important observations was the genetic variation in leaf abscission during flowering. Because leaves contain much higher Ni concentration than stems, loss of leaves after flowering would reduce potential Ni yield. Thus, retention of leaves during early flowering was a selection factor during the recurrent selection program, along with shoot biomass yield, shoot Ni concentration, and plant form (multiple flowering stems, height, etc.).

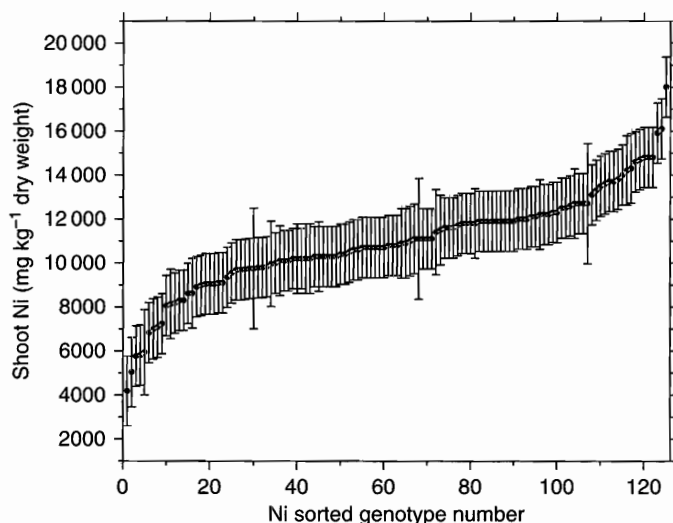


Figure 14.1 Genetic variation among *Alyssum murale* ecotypes in shoot Ni concentration when grown on a uniform test field of serpentine soils in Oregon, USA; points are means of four replications; bars are standard errors (Adapted from Li, Y.-M.; Chaney, R.L.; Brewer, E.; Roseberg, R.J. et al., Development of a technology for commercial phytoextraction of nickel: economic and technical considerations, *Plant Soil* 2003, 249, 107–115 [22].)

Further, it was shown that allowing the cut plants to dry in the sun for several days before baling the biomass did not cause loss of leaf biomass. Although full details have not been released, Viridian LLC (the company which is commercializing this technology) has reported substantial improvement in yield and Ni concentration in improved *Alyssum* cultivars grown on serpentine soils. *Berkheya coddii* was tested under the same field conditions and accumulated only 0.5–1.0% Ni in shoots compared with 2–3% Ni in shoots of improved *Alyssum* cultivars. Similar low shoot Ni was reported for *B. coddii* by Robinson *et al.* [40] even with fertilizer and pH adjustment. It is not evident why field samples of *B. coddii* grown in serpentine soils contain much lower Ni than reported for selected field collections [41]. Perhaps strong nutrient deficiency with very strongly reduced shoot yield caused higher Ni accumulation as reported for *A. murale* in the test by Li *et al.* [42] (Table 14.3). But note the high ability of *A. murale* to obtain adequate phosphate at low P-fertilizer rates. High-Fe serpentine soils require very high P-fertilization rates to support full yields of crop plants [43]. Fertilizer N has repeatedly been shown to increase shoot biomass yields but not substantially reduce shoot Ni concentration [44]. Split application of N-fertilizer will need to be considered to match the growth patterns of the hyperaccumulator species in different climates.

Table 14.3 Effect of soil amendments on trace element accumulation in shoots of *Alyssum murale* grown 120 days on a serpentine soil. For pH treatments, nitric acid was added and allowed to react, then salts were leached from the soil. Gypsum treatments are in Mg ha^{-1} ; phosphate, treatments are in kg P ha^{-1} ; all except control received $150 \text{ mg N as NH}_4\text{NO}_3$, 150 mg K ha^{-1} as KCl and 1 kg B ha^{-1} as H_3BO_3 ^a

Treatment	Final pH	GM ^b -Yield (g/pot)	GM-Ni	GM-Co	GM-Mn	GM-Zn	GM-Fe	Cu
			(mg kg ⁻¹)					
Control								
None	6.56 a	4.1 c	14740. a	34.3 c	56.5 e	63.4 bc	154. b	3.0 cd
Phosphate treatments								
0 P	5.82 e	1.6 d	6250. cd	19.4 ef	62.3 cde	118. a	273. a	2.8 d
100 P	6.24 b	24.5 a	6270. cd	19.9 ef	60.9 cde	59.9 bc	112. cd	3.6 bc
250 P	6.14 bcd	23.2 ab	6810. bc	22.6 def	65.2 cde	60.2 bc	104. d	4.2 ab
500 P	6.16 bc	26.5 a	5690. d	18.1 f	67.2 cde	55.1 cd	92. d	4.0 ab
pH treatments								
Lo	5.42 g	27.4 a	6150. cd	224. a	462. a	63.1 bc	144. bc	4.4 ab
Med-1	5.69 f	26.2 a	6800. bc	50.4 b	132. b	68.7 b	117. bcd	4.6 a
Med-2	5.89 e	27.0 a	5990. cd	28.8 cd	73.1 cd	58.2 bcd	96. d	3.6 bc
As is pH	6.24 b	24.5 a	6270. cd	19.9 ef	60.9 cde	59.9 bc	112. cd	3.6 bc
Gypsum treatments								
0.0 Ca	6.10 cd	19.3 b	7860. b	21.1 ef	55.6 e	49.4 d	87. d	3.1 cd
1.0 Ca	6.24 b	24.5 a	6270. cd	19.9 ef	60.9 cde	59.9 bc	112. cd	3.6 bc
2.5 Ca	6.04 cd	25.2 a	6050. cd	18.4 ef	58.2 de	59.6 bc	87. d	3.8 bc
5.0 Ca	6.03 d	24.2 a	5630. d	24.4 de	78.5 c	63.3 bc	93. d	3.6 bc

^aMeans followed by the same letter are not significantly different ($P < 0.05$ level) according to the Duncan–Waller K-ratio t-test.

^bGM is geometric mean.

Source: adapted from [41], and unpublished data.

One of the most unexpected findings from this research was that although acidification of serpentine soils increased dissolved Ni in soil solution and that extracted by many extracting reagents, Ni accumulation by both *Alyssum murale* and *Alyssum corsicum* was reduced by acidification [42]. In contrast, raising pH of most soils increased Ni concentration and yield [45]. For especially Fe-oxide-rich serpentine soils (~20% Fe), liming above about pH 6.3, however, reduced Ni accumulation. Robinson *et al.* [40] found that *Berkheya coddii* followed the normal pattern of lower shoot Ni concentration with raised soil pH. Thus, use of ammonium acetate extraction to predict phytoextractable Ni from different soils is not appropriate for *Alyssum* species, although it may be relevant for *Berkheya*.

The potential use of chelators to increase Ni accumulation by Ni hyperaccumulators was tested for both *Alyssum murale* and *Berkheya coddii*. Robinson *et al.* [46] showed that added EDTA and NTA reduced Ni uptake significantly. Li, Chen, Chaney and Angle (unpublished results) also found that added EDTA significantly reduced Ni accumulation by *Alyssum murale*. Apparently it is less likely that the added chelator will injure the roots of these hardy plants enough to facilitate metal-chelate accumulation by the plants, in contrast with *B. juncea*.

For phytoextraction to be successful, the forms of Ni in soil must be phytoavailable. Several groups have reported that Ni in serpentine soils was associated with the Fe and Mn oxides or silicates [47,48]. In smelter contaminated soils, Ni was associated with organic matter, Fe oxides, as the Ni-Al layered double hydroxide, and as NiO [49]. NiO has very slow dissolution kinetics [50]; the half-time for NiO dissolution at pH 7 is 20 years. NiO was likely a form emitted from the nickel refinery. A basic test of whether *Alyssum* roots could dissolve this comparatively inert compound showed that *Alyssum* accumulated negligible Ni during a month of root exposure to 1 μm NiO particles [51]. Phytoextraction cannot deal with NiO or similar kinetically inert metal species.

An important characteristic of Ni hyperaccumulators is that they obtain soil Ni from the same pool of 'labile' Ni as other plant species [52,53]. No method is yet known to attack the nonlabile pool of soil Ni except allow time for equilibration to more labile forms. Interestingly, when soil microbes from the rhizosphere of *A. murale* growing on serpentine soils were cultured, several species/strains were found which could induce higher shoot Ni concentration and quantity when the microbe was inoculated into either sterile or non-sterile serpentine soil [54,55]. The mechanism by which soil microbes increase Ni hyperaccumulation remains unknown [56,57]. Several groups have studied changes in the chemistry of rhizosphere soil from hyperaccumulator vs. nonhyperaccumulator species [58,59]. There is little evidence that plants or soil microbes secrete amino or organic acids or other specific Ni chelators which might aid Ni dissolution or uptake.

The most profitable use of Ni-hyperaccumulator biomass is as Ni ore. Chaney *et al.* [12] reported that *Alyssum* biomass ash could enter a Ni refinery at the electric furnace stage rather than going through all the separation and purification steps to separate Ni from rock ores. Biomass energy might offset the costs of crop production, but at this time, no higher value can be obtained from this biomass.

An alternative valuable use of Ni-rich *Alyssum* biomass was recently demonstrated as a Ni-fertilizer for Ni-deficient soils [13]. Ni deficiency of coastal plain soils in Georgia, USA, was demonstrated for pecan trees. Simple sprays of NiSO_4 or a water extract of *A. murale* cured Ni deficiency. Ground *Alyssum* biomass could be applied to the soil and prevent future deficiency. Other old coarse-textured soils managed at pH 6.5 or above may

also suffer Ni deficiency, and the market for inexpensive organic Ni fertilizer could become significant. Other possible crop Ni deficiency is being examined by Wood *et al.* [60].

14.4.2 Soil Cadmium Contamination Requiring Remediation to Protect Food Chains

Extensive areas of paddy rice soils in Japan, China and Thailand have become so contaminated by Zn, Pb or Cu mine and smelter emissions that rice grown on the soils has caused human cadmium (Cd) disease [61,62]. Such locations require Cd remediation in order to continue growing rice for food. Paddy soil remediation in the Jinzu River Valley of Japan used soil removal and replacement, or soil inversion, at a cost of \$2.5 million per hectare [63]. Soil inversion can be successful for rice because paddy rice absorbs Cd only from the surface layer [64]; inversion would not be adequate to alleviate food-chain risk for other crops. Rice readily accumulates Cd in excess of limits (0.4 mg kg^{-1} fresh weight of brown rice) when soils contain as low as $1.5 \text{ mg Cd kg}^{-1}$ [65] due to the soil chemistry of flooded/drained soils, and metal uptake properties of rice (see Chapter 17).

Although many soils are Cd- and Zn-contaminated in western nations, there is little evidence that humans have been harmed by food-chain transfer of soil Cd. How this could occur is discussed in the chapter on Cd and Zn in this book (Chapter 17). Reeves and Chaney [66] and Chaney *et al.* [65] have described how rice transfers bioavailable Cd to humans much better than other crops due to both the Cd uptake relative to Zn and transport of Cd to the grain without Zn being increased, and the Zn and Fe malnutrition induced by subsistence rice diets which promotes Cd absorption in humans.

Other contaminated areas received Cd-rich phosphate fertilizers [67], or high-Cd biosolids [68], or were mineralized with high Cd:Zn ratio marine shale parent rocks (phosphorite source) [69,70]. Crops produced on these soils can contain Cd above guideline or permitted levels. Tobacco also accumulates soil Cd very effectively and like rice may exceed desired Cd levels on acidic soils with low-level Cd contamination [71,72], but few nations have limits on Cd in tobacco. Lack of Cd limits for tobacco is irrational; smoking tobacco contributes strongly to accumulation of Cd in kidney, and hence to Cd risks.

Phytoextraction has been considered a promising method for removing Cd from soils that comprise risk to humans through crop accumulation of soil Cd [73,74]. The species first recognized to hyperaccumulate Cd were *Thlaspi caerulescens* and *Arabidopsis halleri* [5]. However, the 'Prayon' ecotype initially studied did not accumulate Cd well enough to support commercial phytoextraction [75]. Li *et al.* [76] and Chaney *et al.* [77] reported ecotypic variation in Cd accumulation, showing that strains from southern France accumulated about 10-times higher Cd from the same soils as did 'Prayon'. Reeves *et al.* [78] reported on the ecotypic variation in *T. caerulescens* Cd accumulation across Europe, showing more of the pattern that strains from southern France had remarkable ability to phytoextract soil Cd. The southern France ecotypes absorb and translocate Cd more effectively than 'Prayon' apparently due to the root Cd transporter [79]. Li *et al.* [80] eventually obtained a US patent for phytoextraction of soil Cd using such ecotypes and crop management practices to maximize annual removal of Cd.

Even though *T. caerulescens* can hyperaccumulate Cd to more than 1000 mg kg⁻¹ dry shoot biomass when grown on contaminated soils [76,78–84], it has a rosette growth pattern and even at harvest after vernalization is seldom over 30 cm tall. It is not an optimal plant for Cd phytoextraction, and *A. halleri* is even smaller. Research has shown that good management can produce as much as 5 Mg dry biomass ha⁻¹ in the field [75,84–87], although some authors found much lower yields. The 5 Mg ha⁻¹ yield can result from a full year of growth after transplanting including the flowering period before the plants start to drop leaves as seeds are set. It appears that no group has obtained funding to breed improved strains of *T. caerulescens* for field phytoextraction of soil Cd. There appears to be considerable variation in Cd accumulation by plants grown from seeds of a single mother plant, and the source of this variation has not been clarified (Figure 14.2) [74,88]. These findings indicate that normal plant breeding should be able to produce cultivars with even higher Cd phytoextraction ability.

Because this review is focused on more practical aspects of phytoremediation of soil trace elements, it will not include a detailed review of the research on physiology and biochemistry of *T. caerulescens* hyperaccumulation. Several recent reviews cover these topics [89–91].

Acidic soil pH strongly increases Cd and Zn accumulation by *T. caerulescens*. When pH falls to levels which allow Al or Mn phytotoxicity, yields are reduced. Annual

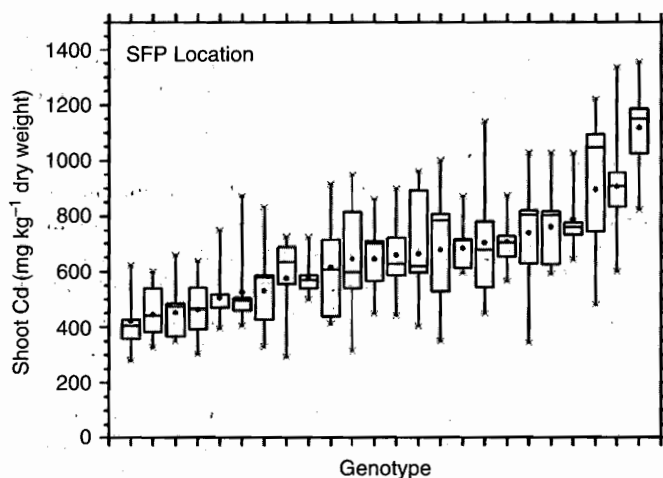


Figure 14.2 Genetic diversity of Cd accumulation by *Thlaspi caerulescens*. Box plots of Cd in *T. caerulescens* genotypes from St. Félix de Pallières (SFP), France; each plot is of results for eight plants grown from seed of a single mother plant (the ends of the box are the 25th and 75th centiles of shoot Cd distribution for a genotype, and the whiskers are the 5th and 95th centiles of shoot Cd; the dot is the mean and the bar the median shoot Cd). Plants were grown for 62 days in pots of soil containing 47.6 mg Cd kg⁻¹ and 821 mg Zn kg⁻¹ due to application of high Cd:Zn biosolids for many years. 'Prayon' ecotype grown in the same experiment contained only 34 mg Cd kg⁻¹ dry weight (Adapted from Perner, H.; Chaney, R.L.; Reeves, R.D. et al., Variation in Cd- and Zn-accumulation by French genotypes of *Thlaspi caerulescens* J. & C. Pres. grown on a Cd and Zn contaminated soil (forthcoming) [290].)

phytoextraction maximum occurs at about pH 5.5–5.7 [81]. The yield responds to N fertilization, but seldom to P, K, or S unless the soil has very low levels of these nutrients [92]. Like *Alyssum* species, *Thlaspi* is adapted to low-phosphate soils and minimal P fertilization will be required for full yield potential. Although higher soil chloride usually causes increased Cd uptake by plants (for example, [67]), if soil pH is lowered to favor Cd uptake, addition of chloride caused little change in plant Cd concentrations [93].

It has been shown that *T. caerulescens* extracts soil Cd from the same 'labile pool' as other plant species rather than extracting nonlabile forms [94–97]. Several researchers have reported that roots of some strains of *T. caerulescens* tend to grow into spots of soil with higher Zn or Cd contamination [98–100]. Haines [100] compared 'Prayon' and 'Bradford Dale' ecotypes and found that only 'Prayon' had the zincophilic root growth behavior. This is another possible contributor to genotypic variation in Zn and Cd accumulation by *Thlaspi*. Others believed that ligands (organic and amino acids) were secreted/exuded by the roots of hyperaccumulator plants and that allowed them to dissolve soil Zn and Cd. But when *T. caerulescens*, wheat, and canola were compared in nutrient solutions, only wheat secreted ligands (phytosiderophores) which could bind significant amount of metals [101].

Several groups have attempted to model uptake of Cd and other elements by *T. caerulescens* and other species. They collected data on the kinetics of Cd uptake by roots, the equilibrium chemistry of Cd in test soils, and the growth of root length over time. These were important first attempts to understand the soil–plant relationships in Cd uptake by hyperaccumulator vs. normal plants [102,103]. There are difficulties in conducting such studies, including measuring Cd uptake at the activity of Cd^{2+} ions in the soil solution. All available evidence indicates that adsorption by the soil limits uptake, so that uptake is diffusion limited. Thus, the actual Cd^{2+} activity at the cell membrane is much lower than the displaced soil solution. The models used to date presume that the entire root length/area is involved in ion uptake, but earlier research indicated that polyvalent cations were absorbed–translocated only by young roots [104]. Thus, continuing root growth to maintain young roots would be very critical to high accumulation. Much remains to understand the mechanism of Cd hyperaccumulation from soils.

What are the alternatives to *T. caerulescens* for soil Cd phytoextraction? Although there have been many papers about other species that can accumulate high levels of Cd, nearly all of them came from study of Cd-salt spiking of test soils without the normal high soil Zn which inhibits Cd uptake by nearly all species. These studies will not be described because they have no application to Cd phytoextraction.

A few plants species today appear to have promise to outperform *T. caerulescens* in Cd phytoextraction, and some can be used in semitropical rice soils where the greatest need exists. A field test of *T. caerulescens* was conducted on the contaminated fields in Thailand with effective Cd removal [105], but climate was a significant limit on growing *T. caerulescens*; monsoon rains ended the first planting in the study. Ae and Arao [106] tested rice genotypes grown under upland conditions and found high enough Cd in shoots, and high enough yield potential to suggest that a technology could be developed. Compared with most other species, rice accumulates high levels of Cd relative to Zn, therefore Cd phytoextraction can be effective even on rice soils with Zn mine waste contamination. They stress that this species grows well in rice soils with tropical weather, and although the potential Cd concentration in shoots is far lower than *T. caerulescens*, in

these soils and climate, *T. caerulescens* does not grow well if at all. The Japanese team tested more genotypes of rice, and conducted greenhouse and field tests of Cd phytoextraction from contaminated fields that needed remediation to lower Cd in rice grain [106–108]. The most complete demonstration to date was two years of using rice to remove soil Cd, followed by growing food rice on the phytoextracted and untreated soils [109]. The test was clearly successful; the soil contained 1.63 mg Cd kg⁻¹ and 134 mg Zn kg⁻¹, and the initial pH was 5.6 (water); phytoextraction removed 883 g Cd ha⁻¹, lowering soil Cd by 38%; rice grain Cd was lowered 47%. Rice grain contained 1.02 mg Cd kg⁻¹ on the control treatment, and 0.54 mg Cd kg⁻¹ on the phytoextracted treatment with the best phytoextraction cultivar. Normal rice production practices were used. In their present method, they grew the 'Chokoukoku' rice with flooding until flowering and the shoots contained 0.42 mg Cd kg⁻¹ on July 7, but the fields were drained at flowering and at harvest on October 19 the rice shoots contained 70 mg Cd kg⁻¹. This shows again the key role of field drainage in Cd accumulation by rice, and that the combination of high biomass yield and moderate Cd accumulation gave successful Cd phytoextraction. Murakami *et al.* [110] had earlier shown a similar reduction in soybean Cd after using rice to remove soil Cd. Because the allowable levels of Cd in rice in Japan have been lowered from 1.0 mg kg⁻¹ to the new Codex Alimentarius standard of 0.4 mg Cd kg⁻¹, Murakami *et al.* [109] estimate that more than 40 000 ha of rice paddy soils in Japan will require Cd remediation.

In addition to the promising results for rice, others have examined plants growing on Cd+Zn mineralized or contaminated soils to see if other natural Cd hyperaccumulators with high yields could be found. Chen *et al.* [111] suggested that co-cropping *Athyrium yokoscense* and *Arabis flagellosa* might give useful Cd removals, but both species have low yield potential and lack high tolerance of Zn. Yang *et al.* [112] found that *Sedum alfredii* Hance collected at a metal-rich mine waste area in China hyperaccumulated Cd and Zn and did physiological research on this species. It is difficult to obtain seeds from *S. alfredii*, but the growth form is taller than *T. caerulescens*, and the plant tolerates semitropical climate. Deng *et al.* [113] found considerable genetic variation in metal accumulation and tolerance by *S. alfredii* (from 1.1 to 1051 mg Cd kg⁻¹ shoot biomass), so there may be hope of developing a higher biomass Cd hyperaccumulator *S. alfredii*. Xu *et al.* [114] found that another *Sedum* species, *Sedum jinianum*, may also be able to phytoextract Cd from tropical soils. *Sedum* lacks the exceptional Cd accumulation of the southern France ecotypes of *T. caerulescens*, but they can grow in tropical environments. A tropical fruit, carambola (*Averrhoa carambola* L.) (star fruit), was also found to accumulate relatively high levels of Cd in shoots, and by growing plants at high density for shoot harvest (compared to normal orchard density), the authors believed that phytoextraction using carambola could be useful [115,116]. Phaenark *et al.* [117] examined native plants growing on Zn mine waste contaminated or Zn-Cd-mineralized soils in Thailand and reported four new Cd hyperaccumulator species based on field data. The species identified and shoot Cd concentrations were: *Chromolaena odoratum* (a perennial shrub), 166 mg kg⁻¹; *Gynura pseudochina* (an annual herb), 458 mg Cd kg⁻¹; *Justicia procumbens* (an annual herb), 548 mg Cd kg⁻¹; and *Impatiens violaeiflora* (an annual herb), 212 mg Cd kg⁻¹ dry weight. These authors felt that *J. procumbens* had the greatest promise for practical phytoextraction because of its growth characteristics, but they have not yet characterized the farming of these species, effect of pH and fertilizer application, and so

on, on Cd phytoextraction effectiveness. Presently, reproduction of this species is based on stem cuttings, which would make its use more difficult than that of a seed plant (P. Pokethitiyook, Mahidol University, Bangkok, Thailand; personal communication, June, 2009). They also noted that *C. odoratum* grew year-round as opposed to growing only during the rainy season, so it may also offer promise. Only controlled field testing over several years will show how useful these species might be.

The promising phytoextraction technologies for soil Cd in the most common contaminated soils with 100 times more Zn than Cd include: (1) improved domesticated *T. caerulescens* from southern France with pH management, for perennial culture; (2) domesticated *Sedum alfredii* or other *Sedum* species; (3) high Cd accumulating rice cultivars; (4) one of the newly identified Cd hyperaccumulators from Thailand; or (5) if biomass energy production is profitable, using willow to remove Cd while producing energy. Until government requires soil Cd remediation, it is likely that no practical technology will be developed.

14.4.3 Phytoextraction or Phytovolatilization of Soil Selenium

Soils can be geochemically enriched or contaminated anthropogenically in selenium (Se) such that the Se comprises a health risk to humans [118] or wildlife [119,120]. The Kesterson reservoir case in the Central Valley of California, USA, stimulated research because fish and birds suffered severe birth defects from excessive Se accumulation [119,121]. These drainage water evaporation pond aquatic ecosystems allowed Se biomagnification and much harm, though not directly caused by Se-mineralized cropland.

The existence of Se accumulator plants was well known [122] when studies testing different plant species for their ability to accumulate or exclude Se as possible solutions to Se enriched soils were initiated [122–126]. Banuelos [9] noted the economic limitations of using hyperaccumulator plants to achieve phytoremediation of Se-contaminated soils. He examined production of nonfood crops, and of foods with higher than normal Se levels to be sold as improved Se-rich crops, and production of forages with enough Se to replace Se supplements normally added to livestock diets. Banuelos and Mayland [8] tested the efficacy of Se supplementation using the Se-rich crop plants and found them to be a valuable Se supplement. Perhaps the best use of Se-rich biomass is to recycle the Se into livestock diets in place of virgin Se. By producing canola (*Brassica rapa* L.) for oil (food or biodiesel) and its deoiled seed press cake which could be used for Se-enriched livestock feed or organic crop Se-fertilizer, the grower could obtain value for producing the crop while reducing Se release to irrigation drainage waters [127].

An alternative phytoremediation approach, phytovolatilization, was also considered for remediation of Se-rich soils and water [128]. Both microbes in the rhizosphere and plants can produce dimethyl selenide which is volatilized from soils, roots and leaves. Plant shoots were not required to achieve useful phytovolatilization of soil Se [129]. Hansen *et al.* [130] attempted to convert this into a technology irrigating Se-rich wastewater on wetlands to remove Se from industrial wastewater, but found that more Se was accumulated in the soil than was phytovolatilized, so long-term risk from the treatment site had to be managed at higher cost.

An important principle of Se accumulation by plants is the normal competition between sulfate and selenate for root uptake. Bell *et al.* [131] found that although sulfate inhibited

uptake of selenate by crop plants, it did not inhibit selenate uptake by the Se hyperaccumulator species *Astragalus bisulcatus*. This principle was extended by White *et al.* [132], who suggest that different selenate selectivity of the high affinity sulfate transporter in roots is the fundamental characteristic of Se accumulator plants. Inversely, when Banuelos [133] tested growing crop plants such as canola and broccoli on Se-rich soils, he found one could depend on the high sulfate level in the same soils to limit Se accumulation by the crops, allowing them to be marketed as Se-rich foods.

Thus, several approaches to phytoremediation of soil Se have been reported. In one, crop plants are grown which accumulate moderate levels of Se and might be sold as Se-enriched foods for extra value. In a second, crops are grown to support phytovolatilization and reduce Se in drainage waters. And in a third, phytoextraction using hyperaccumulator species may remove soil Se over time and produce soils which no longer threaten food safety or drainage water due to excessive Se. Phytoextraction of soil Se is complicated because part of the soil Se is in organic forms and in soil organisms, which are not immediately available for plant uptake.

Because Se hyperaccumulators were well known when problems arose in California [122], researchers tested growing *Astragalus* species to remove Se [123,131]. The concentration and biochemistry of Se in *Astragalus* vary with species, accession, tissue, and season [134–136]. Hyperaccumulators make a methyl derivative of selenocysteine which prevents it from entering proteins, protecting the plant from Se toxicity experienced by nearly all other species. Several researchers have noted the difficulty in growing *Astragalus* species (difficult germination of hard seed; one needs to treat seed with sulfuric acid to obtain germination) and looked at alternative Se hyperaccumulator species [137,138]. Feist and Parker [139] examined *Stanleya pinnata* collected from different sites and found considerable ecotypic variation in Se accumulation when grown in a uniform test. Improved phytoextraction cultivars might be available through breeding. Galeas *et al.* [140] reported seasonal variation in Se concentration in shoots of Se hyperaccumulators which could be used to determine the optimum time for harvest.

The alternative to development of hyperaccumulators was to produce transgenic plants which tolerate Se better than crop plants [141]. Eventually a field trial of transgenic *B. juncea* confirmed that they could achieve improved Se phytoremediation in the field [142]. The transgenic plants do not yet have the selective uptake characteristic of Se hyperaccumulators, and thus do not accumulate such high levels. On the other hand, they are easier to grow than the hyperaccumulators and offer the biodiesel plus oilseed feed supplement as economic products of cropping to alleviate Se risks.

Research on Se hyperaccumulator plants has shown that Se can be accumulated in specific tissues rather than dispersed evenly [143], and that Se reduces predation by leaf-eating insects [144]. This finding supports the 'defense' hypothesis regarding evolution of hyperaccumulators. Interestingly, Freeman *et al.* [145] found that mutant Diamondback moths (*Plutella xylostella*) had evolved on areas where *Stanleya pinnata* was dense and the moths tolerated high levels of dietary plant Se without injury; further, they found wasps which parasitized the moths which were also tolerant of high Se. Evolution generated hyperaccumulators, and then generated insects which tolerated the hyperaccumulators.

Plant Se levels as low as 38 mg kg^{-1} dry weight reduced predation by prairie dogs [146], showing evidence that hyperaccumulation can stop mammals as well as insects and microbes from harming plants with this property. Plants emitting $(\text{CH}_3)_2\text{Se}$

have a strong garlic odor, which may cause livestock to avoid consuming them unless little other forage is available [122]. Chronic diets with $>3 \text{ mg Se kg}^{-1}$ are considered a risk to livestock [147]. Thus, moderate Se accumulation gave the Se-hyperaccumulators protection against insect predators.

14.4.4 Phytoextraction of Soil Cobalt

Brooks *et al.* [148–150] reported unusual African plants (*Haumaniastrum*, *Aeolanthus*) growing on copper (Cu) and Co (cobalt) mineralized soils that hyperaccumulated Co, giving hope that Co phytoextraction could be developed. Ni hyperaccumulators they collected in that same time period had much poorer Co accumulation from serpentine soils and had low tolerance of absorbed Co [151]. Li *et al.* [41] reported that *Alyssum murale* could accumulate some Co from serpentine soils, and that plant Co concentration increased with soil acidification in contrast with Ni accumulation, which decreased with soil acidification. Keeling *et al.* [152] examined Ni and Co phytoextraction using *Berkheya coddii*, and found that Co phytotoxicity limits the use of *Berkheya* in Co phytomining. Malik *et al.* [153] tested the *Alyssum* Ni hyperaccumulators, *Brassica juncea*, and known Co accumulator species *Nyssa sylvatica*. When Ni was not present at the normal 10-fold ratio to Co of serpentine soils, *Alyssum* species accumulated and tolerated over $1000 \text{ mg Co kg}^{-1}$ dry shoots, but Ni strongly inhibited Co accumulation by the tested species. *B. juncea* suffered strong Co phytotoxicity when *Alyssum* and *Nyssa* grew normally.

The initial controlled greenhouse test of Co uptake by these species used Co-salt-amended potting media and measured high Co accumulation in several species [154], so their ability to hyperaccumulate Co ($>1000 \text{ mg kg DW}$ (dry weight)) under this laboratory condition was established. However, subsequent research on the African Co accumulator species has raised questions about the analysis of these plants due to mineralized soil contamination of leaf samples [155]. Recent studies using mineralized soils showed weaker plant accumulation of Co and Cu [156].

For the *Alyssum* phytoextraction technology, raising soil pH to maximize Ni phytomining reduced Co accumulation [45], but at the end of Ni phytomining, the soil could be acidified without much risk of Ni phytotoxicity to allow more effective Co phytomining. Alternatively, *Alyssum* species could be grown on Co-mineralized or Co-contaminated soils. A striking finding was reported by Tappero *et al.* [157]: Co was not stored in leaf vacuoles similarly to Ni storage in *Alyssum murale*; Co remained within the xylem system or cytoplasm or exited leaves at the tips, forming a deposit on the leaf surface. Because Co is 3–4 times more valuable than Ni [158], Co phytomining could be cost-effective if plants and agronomic practices were developed to maximize annual biomass Co quantity.

14.4.5 Phytoextraction of Soil Boron

One example of effective boron (B) phytoextraction has been reported by Robinson *et al.* [159]. A site in New Zealand where wood was treated with boron chemicals had a large pile of waste materials from which B had leached to groundwater. Boron levels exceeding allowed limits were measured in nearby surface water. Robinson *et al.* tested growing hybrid poplar to evapotranspire water and limit off-site movement of the B-rich plume of

groundwater, and irrigated groundwater onto the waste pile to supply water during dry periods of the year. The poplar leaves accumulated high B levels, more than 1000 mg kg^{-1} DW, much higher than the woody tissues. They proposed harvesting the trees on a three-year cycle, with the crop used for energy production or stock fodder, or even harvesting the leaves as a B-rich organic-fertilizer. Although sale of leaves as organic B-fertilizer has promise, the wood treatment company chose to simply prevent stream contamination.

High B levels in Se-contaminated irrigation drainage waters limits species which can tolerate soils managed to obtain phytoremediation of Se in the drainage waters. Thus Banuelos [133] looked at preventing Se from entering the drainage waters by growing crops which phytovolatilized Se. The high B, salinity, and Se in the drainage waters put severe limitations on treatment alternatives.

14.4.6 Phytovolatilization of Soil Mercury

Mercury (Hg) is a complicated element for phytoremediation. First, normal plants do not absorb and translocate important amounts of Hg from soils through the xylem to shoots; most shoot Hg arrives by volatilization from soils [160–165]. Photovolatilization is very important in releasing Hg vapor from soils, so heavy plant cover can strongly inhibit photovolatilization [166]. Roots and rhizosphere microbes can reduce Hg^{2+} to Hg^0 with volatilization from the soil surface, but hardly from below a few millimeters into a soil [167].

There are no land plants which hyperaccumulate Hg when growing in a Hg-rich soil except by capturing volatilized Hg^0 more effectively than other plant species growing on the soil, for example, mushrooms [168], but such fungi would not provide a convenient species for phytoextraction of Hg. Many studies of Hg uptake fail to control Hg^0 volatilization from the surface of the growth medium, which allows volatilized rooting medium Hg to reach the shoots and confound the interpretation of the uptake to shoots. Plant transpiration of root-absorbed Hg is a minor process [163], although some Hg does reach shoots by the xylem.

A team lead by Meagher developed transgenic plants expressing bacterial mercuric reductase (MerA), and organic-mercurial lyase (MerB) to see whether transgenic plants could be developed to achieve phytoremediation of soil Hg [169,170]. Effective expression of these genes in plants required changing some codons from those used in bacteria. Meagher *et al.* showed this approach allowed plants to phytovolatilize Hg from Hg^{2+} in soils when MerA was expressed in plants, and from CH_3Hg^+ when both MerA and MerB were expressed. They expressed the genes in several plant species to seek species for practical soil Hg phytoremediation [171–173]. Although the technology was scientifically successful, it was not accepted by regulatory agencies or commercial users because Hg would be released to the air from soil, and that Hg would eventually return to earth and possibly cause Hg risk where it was deposited. Subsequently, Meagher *et al.* attempted to develop plants which accumulated Hg inside the plant by expressing phytochelatin synthase [174]; although this increased As and Hg tolerance, it did not achieve accumulation of Hg in the plant biomass. In addition, their test did not prevent Hg volatilized by treated roots from reaching the shoots and cannot be considered definitive. The last advance in Hg phytoremediation was a demonstration that expression of the organic mercurial lyase

secreted through root cell walls could increase plant tolerance of organic mercurials, and increase Hg volatilization better than general expression of the transgene in the plant [175].

Soil Hg comprises risk through soil ingestion and through aquatic ecosystem biomagnification of methyl-Hg from sediments. Large areas of soil are Hg-enriched where Au or Hg mining occurred historically. Such soils may be barren and eroding into streams where the Hg could cause much greater risk. Bare soils rich in Hg are highly subject to photovolatilization. Phytostabilization of such soils using combinations of organic matter-rich nutrient sources and required alkalinity could strongly reduce Hg release to the environment.

Moderately contaminated soil could be phytoremediated using the MerA-MerB transgenic plants described above. The likelihood of local deposition of the phytovolatilized Hg^0 is very small, and the benefit to society is large. The dig and haul alternative is much more expensive and could disperse the contaminants. The poplar, rice and other transgenic Hg remediation crops offer much promise.

14.4.7 Induced Phytoextraction of Soil Gold

Gold (Au) may be a cost-effective induced phytomining opportunity. Economic Au phytomining first requires standard ore mining and grinding, then placing the ground ore over plastic membranes to prevent leachate loss so that cyanide, thiocyanate or thiourea used to induce phytoextraction can be irrigated on the soil to promote Au uptake by growing plants [176–178]. Others have looked for formation of gold nanoparticles in the plant biomass because the nanoparticles may have higher value for use as catalysts, and other uses, than the gold content alone [179] and tested diverse plants to phytoextract soil Au [180,181]. Australian researchers estimated that Au phytomining could make more return per hectare than Ni phytomining [182], so this research topic remains active.

14.4.8 Induced Phytoextraction of Soil Lead

In 1995, Kumar *et al.* [29] reported accumulation of more than 1% lead (Pb) in shoots of some genotypes of *Brassica juncea* grown in sand culture supplied soluble Pb with no phosphate or sulfate in the nutrient solution. These conditions allowed high uptake of Pb and severely harmed the plants, which were then harvested and analyzed. On the basis of these data the authors obtained a patent for phytoextraction which they believed covered all plants and soil management for phytoextraction. Fortunately, their patent excluded the 'low-yielding' natural hyperaccumulator plants and did not stop R&D on practical phytoextraction.

Subsequently the Rutgers University/Phytotech team tested Pb uptake from contaminated soils and found very low uptake by the same genotypes of *B. juncea* that worked so well in nutrient solution, and then developed the addition of chelating agents to induce phytoextraction of Pb from soils [183,184]. The patent was licensed to a Phytotech, Inc. which conducted Pb phytoextraction field tests. Further research showed the leaching of Pb and other metal chelates from treated soils, and Pb contamination of groundwater. The company eventually went bankrupt, and it is no longer possible to obtain permits to conduct field scale induced phytoextraction of soil Pb in the United States or European Union. It is very unfortunate that many researchers were misled by these studies to believe

that Pb phytoextraction might be practical. Literally hundreds of papers have since been published on Pb phytoextraction with different plant species and different chelating agents. None have demonstrated a cost-effective and environmentally acceptable phytoextraction technology for soil Pb.

It had long been known that if plants grown in soil or nutrient solution were deficient in phosphate and no phosphate fertilizer was added, the plants could accumulate and translocate high amounts of Pb (for example, [185]). The presence of phosphate causes formation of an insoluble Pb-phosphate compound in roots. When adequate phosphate exists in the soil to produce a normal crop yield, even $3200 \text{ kg Pb ha}^{-1}$ applied to a field soil caused little increase in Pb in *Zea mays* L. [186]. Cotter-Howells *et al.* [187] showed that plant roots/microbes caused formation of the nonphytoavailable Pb compound chloropyromorphite when growing in Pb-rich soils. Thus, normal soil and plant Pb chemistry prevents plant Pb uptake-translocation to any meaningful extent. Two minor accumulators ($<1000 \text{ mg Pb kg}^{-1}$) of Pb (hemp dogbane, *Apocynum* sp. and common ragweed, *Ambrosia* sp.) were reported by Cunningham and Berti [188], who collected and analyzed plants growing on a Pb-contaminated field; other species contained $<10 \text{ mg Pb kg}^{-1}$ after washing to remove soil and dust from leaves – a normal level for most plants growing in such strongly contaminated soil.

Valuable studies conducted by Blaylock *et al.* [183], Huang *et al.* [184], and Cooper *et al.*, [189] clarified the need for adding chelating agents to soils in order for Pb to be accumulated by plants. With added chelators, several species could accumulate over 1% Pb in dry shoots [190]. In general, the plant is grown for some period to give biomass with effective evapotranspiration; then the chelating agent is applied in soluble form to the surface of the soil. Within a week or two, the plants are dead from multiple metal or chelator toxicity and ready to harvest. Research was conducted which showed that intact Pb-EDTA did reach the shoots of several species [191,192]. And basic studies showed that EDTA had to be in excess of Pb or other strongly chelated cations in the nutrient or soil solution for the EDTA to stimulate high uptake of Pb [191–193]. It is believed that free EDTA has to harm the root cell membrane enough to make it leaky, and then the solution containing Pb-EDTA (and other metal-EDTA chelates) enters the transpiration stream and rises to plant shoots [194]. Although Pb-EDTA may have low phytotoxicity to tested species, other metals and free EDTA strongly affect plant health when high levels are supplied to soils for induced-phytoextraction.

Many soils have mixed metal contamination such that uptake of Zn, Cu, and other ions are also greatly increased by chelation treatment because the chemistry of the specific chelating agent utilized controls which soil elements are dissolved [183,195,196]. Discussion of chelation equilibria can be found in Nowack [197], and Parker *et al.* [195]. Chelator selectivity among elements, surface area binding of the metal in soil, and activity of metals in the soil control which metals are dissolved and how rapidly they are dissolved.

In tests we conducted with *B. juncea* and Ni and Co accumulation, the growth habit of the plant seemed ill-suited to phytoextraction [153]. *B. juncea* is normally sown in the fall, grows over winter, and flowers when long days return in the spring. If the plant is started in the spring, it begins to flower in about 4 weeks, greatly limiting biomass and potential transpiration needed to accumulate much Pb-EDTA from soil. Some *B. juncea* phytoextraction research used 10-hour days to prevent conversion to flowering growth

(vernalization) [193]. Banuelos [126] initially studied *B. juncea* and collected local strains of this species in India because it might be useful in Se phytoextraction. Others chose to use it for Pb and many other elements for which there was never any evidence to suggest this species could be useful.

Many papers have since discussed concern about metal leaching when chelating agents and detergents were applied to induce phytoextraction [196,198–201], and some have shown strong experimental evidence of the leaching [202–208]. There can be little question that metal leaching precludes field application of EDTA-induced metal phytoextraction. It was noted above that cyanide-induced Au phytomining could be conducted commercially only because the 'soil' or ground ore was placed on a plastic membrane to conduct cyanide heap-leaching of Au before using plants to phytoextract some of the remaining Au. Open field cyanide leaching is not acceptable, similarly to open field EDTA-induced phytoextraction.

Other chelating agents were tested using different timings of chelator addition and split applications, combined with other treatments (for example, [183]). In particular, EDDS (ethylenediamine disuccinic acid) was tested because it is more rapidly biodegraded and hence less likely to cause unacceptable leaching. Testing showed that leaching was still a problem under any condition which allowed EDDS addition to stimulate Pb uptake [196].

Another important consideration of chelator-induced Pb phytoextraction is the cost of the added chelator. Chaney *et al.* [12] obtained the price of truckload quantities of EDTA (\$4:30 kg⁻¹ in 2000) and calculated the cost of applying 10 mmol EDTA kg⁻¹ soil for 1 ha–15 cm deep (2×10^6 kg), the dose required for optimal Pb phytoextraction [183,184]. One application of EDTA would cost \$30 000 ha⁻¹. When EDTA is added at these levels, one must wait before planting *B. juncea* again because of phytotoxicity until the metal chelates leach or are biodegraded. Thus this method is very expensive as well as comprising risk to groundwater contamination if liners are not used. With the highly effective *in situ* inactivation of soil Pb described below, it seems clear that inactivation of soil Pb is the more desirable approach for soil Pb remediation.

14.4.9 Phytoextraction of Soil Arsenic

Soil arsenic (As) is a risk to children through inadvertent soil ingestion, and to all ages of humans through dietary exposure from rice accumulation of As from contaminated soils, especially those irrigated with As-rich water. Current calculations by the US-EPA indicate that the limit for soil As should be 4.3 mg kg⁻¹ to protect against 10⁻⁵ increased lifetime cancer risk based on soil ingestion. This concentration is within the normal range of soil As levels for background uncontaminated soils (5th and 95th centiles = 2 and 12 mg As kg⁻¹) [209], so it is not clear that the US-EPA limit is justified. In practice, soil cleanup to 20 mg kg⁻¹ has been selected for several US Superfund sites in urban areas. Because Department of Defense activities during World War I caused soil As contamination in the Washington, DC, area, and US-EPA designated the site a Superfund site, soil remediation was conducted by Edenspace, Inc. under contract. Edenspace licensed use of the As hyperaccumulator fern *Pteris vitatta* from the University of Florida [210] and conducted field operations. Because soils contained in the order of 40 mg kg⁻¹, with localized higher

concentrations, Edenspace used a combination of excavation of highly contaminated soil, and tillage prior to phytoextraction to reduce hot spots. This is either an advantage of phytoextraction compared to soil removal, or a problem with phytoextraction. Tillage is both normal and required for commercial phytoextraction. When the risk and clean-up goal is based on average surface soil As concentration, alleviating hot spots clearly reduces risk. For risk to occur, children must ingest the soil from the soil surface, so reducing As levels in the surface soil is the valid goal and tillage contributes to this goal. After a few years of As phytoextraction, Edenspace was able to reduce soil As to regulatory limits (M. Blaylock, Edenspace Inc., personal communication, June, 2009). In another field test of As phytoextraction, the point-to-point soil As variation of the 30.3-m² plot was so great that two crops of *P. vitatta* did not significantly reduce soil As concentration while they reduced average soil As from 190 to 140 mg kg⁻¹ [211].

The As hyperaccumulator fern, *P. vitatta*, was discovered by Ma *et al.* [210] by analyzing many plant species growing at an As-contaminated site. They have done extensive research to develop this technology, including study of the chemical form of As in soil, and the effect of fertilizers and pH management on phytoextraction. Interestingly, although As enters most plants on the high-affinity phosphate transporter [212], and solution phosphate inhibits As uptake, phosphate did not inhibit As accumulation by *P. vitatta* [213]. Others have studied fern variation in As accumulation, finding other *Pteris* species, and other fern genera, that contain As hyperaccumulators [214–216]. Wang *et al.* [217] examined variation of As accumulation by ferns collected at different locations in South China and found genotypic variation within *P. vitatta* that could be useful in breeding improved cultivars.

The effect of rhizobacteria from the rhizosphere of field-grown ferns on As uptake, translocation, and fern yield in high As solutions and soils was tested [218]. Although rhizobacteria increased shoot As in nutrient solutions, they had no effect when used to inoculate ferns grown in sterilized soils. Mycorrhizae were found to significantly increase As and P phytoextraction by *P. vitatta* [219], but the benefit of inoculation in nonsterilized soils has not been reported.

The fern appears to tolerate high biomass As levels because it can store the As in vacuoles or make phytochelatins to bind part of the As within cells [220,221]. Only about 1–3% of total shoot As was found to be bound by phytochelatins [222], so the significance of As binding by phytochelatins is uncertain. Although the roots absorb arsenate, arsenite is the dominant form of As in the leaves [223]. Several papers suggested that reduction occurred in the shoots, but a careful test by Su *et al.* [224] measured As species in bleeding xylem exudate of *P. vitatta* and showed that arsenite was the dominant form present, confirming that reduction largely occurred in the roots. Previous tests used pressure expression of xylem sap and obtained very different and apparently incorrect results [225]. Arsenic has localized distribution within the fern plant (tissue concentration is much higher in young than old fronds) [226,227] and appears to be stored in epidermal cell vacuoles similar to other hyperaccumulators with other metals, and in trichomes [228].

Although *Pteris vittata* can give effective shoot biomass yields, it does not tolerate cold environments, and is difficult or time-consuming to establish; transplants are used after starting the fern propagules. Thus development of a transgenic seed plant with field As phytoextraction potential could be important for solving soil As contamination problems [174]. Dhanker *et al.* [229] found that if they transferred genes for arsenate

reductase and γ -glutamyl-cysteine synthetase into plant species, plant tolerance of As was greatly increased [174]. These species were still not as able to hyperaccumulate As from soils as well as the natural fern hyperaccumulators, but if the arsenate reductase in *Arabidopsis thaliana* roots was silenced by RNA interference, arsenate was more freely translocated to shoots (a 10- to 16-fold rate of wild type) [230]. Apparently the root uptake or arsenate reduction characteristics and/or arsenite translocation characteristic of the ferns must also be transferred into transgenics in order to obtain seed plants with effective As hyperaccumulation.

Arsenic is also a potential risk through drinking water, and with recent lowering of drinking water As limits, many water producers must find a way to reduce As in their product. Chemical removal can be expensive. Elless *et al.* [231] tested use of *P. vitatta* to phytoextract As from raw drinking water and found they could reduce water As to $< 2 \mu\text{g l}^{-1}$, well below US limits ($10\text{--}15 \mu\text{g l}^{-1}$) for As in drinking water.

Yan *et al.* [232] demonstrated that incineration of As-rich biomass caused volatilization of the plant As, but that should have been expected. Copper smelters would emit large amount of As if not required to control all emissions, which is readily achieved using existing technology. If biomass energy production could reduce the cost of As phytoextraction, the incineration could be conducted using proper exhaust treatment. As noted above, to date As-rich phytoextraction biomass has been disposed in landfills.

In situ inactivation or phytostabilization of soil As has been demonstrated by several research groups. Reduced phytoavailability was shown for Fe additions to soils [233]. Reduction of *in vitro* bioavailable or bioaccessible soil As has been demonstrated by Subacz *et al.* [234], by Beak *et al.* [235], and by Smith *et al.* [236] by adding Fe to soils. By comparing the EXAFS As speciation results with bioavailability results from pig feeding studies, Beak *et al.* [235] showed that As associated with Fe was essentially nonbioavailable to young pigs. Thus *in situ* treatment may be an effective treatment for soil As risks. With the extensive contamination with As of irrigated rice soils in Bangladesh, some As remediation solution is needed to limit As uptake into rice grain and to prevent future risk to children from ingestion of these soils [237]. Similarly, the extensive area of As-rich soils in south-east England attributed to historic tin mining requires As remediation [238].

14.4.10 Phytoextraction of Other Soil Elements

Phytoextraction of a few other elements has been studied to some extent. Soil thallium (Tl) may comprise risk to humans at some locations where local industry emitted Tl, or major Pb smelters contaminated large areas, so studies were conducted comparing vegetable crop accumulation of Tl. Green cabbage accumulated much higher Tl levels than most other vegetable crops [239,240]. Fortunately, unusual hyperaccumulator species were discovered for Tl and they accumulate high plant Tl from soils with moderate to high Tl contamination [241,242]. The strong hyperaccumulator, *Iberis intermedia*, accumulated Tl in the leaf vascular system rather than in vacuoles or trichomes as seen for some other hyperaccumulated elements [243].

Cesium (Cs) risk is most important for the radionuclide (^{137}Cs) which is spread rapidly after nuclear fission releases. It was well known that Cs could be easily fixed within soil clay, and that high soil K inhibited Cs uptake by plants. Substantial species variation in Cs

accumulation from soils has been demonstrated, but no special hyperaccumulator type was identified [244]. Researchers compared a number of species relative uptake of Cs and found that red root pigweed (*Amaranthus retroflexus* L.) accumulated much higher shoot Cs levels than other studied species [245,246]. Redroot pigweed bioaccumulated Cs from soils into shoots, while most other species accumulated Cs in roots. This species also has a relatively high potential shoot yield for phytoextraction. Depending on the extent of contamination, the clean-up period could be longer than desired [247]. Dushenkov *et al.* [248] tested using sunflower and *Brassica juncea* to phytoremediate ^{137}Cs contaminated soils near the Chernobyl nuclear site, with limited success. An alternative approach was tested in Belarus, growing canola on the ^{137}Cs -contaminated soils so that the canola oil could be used for biodiesel and the seed meal used for feed or fertilizer. Most elements do not follow the oil during crushing of oilseeds, and ^{137}Cs was hardly present in canola oil from seeds with lots of radioactivity. Here again the economic solution may not support phytoextraction, but the principles of soil-plant science for each contaminant are the basis for public decisions. The original plan was to use the canola oil as biodiesel, but when it was found to have such low ^{137}Cs activity, it was all used for human food.

Uranium (U) phytoremediation research has taken several directions. In one, plant roots were used to remove U from contaminated groundwater in a process called *rhizofiltration* [249]. After testing several species in the laboratory, an innovative field trial was conducted using alfalfa sprouts in plastic frames over which well water containing excessive U was irrigated; this rhizofiltration was quite effective in removing U, but not as inexpensive as the traditional removal using resins [249]. Natural uptake and translocation of U by plant roots are quite low, so Huang *et al.* [250] tested using chelator-induced phytoextraction for soil U; citrate increased U dissolution and uptake substantially. Whether this could be cost-effective has not been reported, but the technology has not been commercialized. Citrate addition can increase leaching of U, but because citrate is readily biodegradable, the potential for adverse impact is less than seen for addition of EDTA.

Molybdenum (Mo) and tungsten (W) can accumulate in soils to phytotoxic or zootoxic levels which require remediation. Certain legumes are able to accumulate much higher levels of Mo than most grasses [251], but no effective phytoextraction technology using legumes which accumulate Mo has been reported. Treatment of the soil with phosphate or vermiculite raised Mo uptake for phytoextraction, while other treatments lowered plant Mo and alleviated potential for Mo zootoxicity [252]. An overview of soil Mo risks and management strategies was reported by O'Connor *et al.* [253].

14.5 Phytostabilization of Zinc-Lead, Copper, or Nickel Mine Waste or Smelter-Contaminated Soils

It is evident from the previous discussion that phytoextraction is not practical for Zn and Pb, which are among the most common soil contaminants of public concern. Zinc, Cu, and Ni are the most common cause of soil metal phytotoxicity for most plant species and cause barren areas where acidic soils are contaminated. Often erosion of the contaminated soils, or the presence of low nutrient mine wastes causes severe infertility as well metal stress. Lead is more important as a risk to children and wildlife health through ingestion of soil

than through plant uptake. Lead is included in this discussion because most Pb and Zn ores are mixed and include Cd as well, so remediation of Pb and Cd must also be achieved when Zn phytotoxicity is remediated. Research has increasingly shown that combinations of soil amendments to make the soil calcareous and fertile can remediate these risks and allow effective soil cover which protects wildlife. It is not certain that sensitive crop plants will succeed on such *in situ* remediated soils, but many common grasses and legumes do very well after soils have equilibrated.

In situ phytostabilization was first considered as an alternative to establishing metal tolerant ecotypes of grass [254] which had been studied and developed by Bradshaw *et al.* [255]. The 'Merlin' red fescue (*Festuca rubra*) they developed does very well on fertilized Zn toxic soils compared with other species (for example, [26]), but such plantings require regular fertilization to persist. In the absence of other legumes and plant species with similar genetic tolerance to soil metals, an ecosystem cannot develop. There are no legume ecotypes with high metal tolerance needed to supply N for other species on Zn phytotoxic acidic soils.

Gadgil [256] considered using biosolids and municipal solid waste compost coupled with metal-tolerant grass ecotypes to revegetate barren soil contaminated by Zn, Ni, and Cu. In some cases she included alkaline pulverized fuel ash from coal-fired power generators, which raised soil pH and aided in reducing metal phytoavailability.

Research has since shown that organic matter, Fe and Mn oxides, and phosphate in biosolids and other soil amendments can increase metal sorption or precipitation in contaminated soils, and aid in revegetation [27,257–259]. Combined with limestone to make the amended soil calcareous, biosolids or composts can provide effective revegetation and limit plant uptake of metals to protect wildlife food chains [260]. The effective control of Pb uptake and bioavailability from ingested soil was demonstrated by Ryan *et al.* [261]. Depending on the soil amendments, Pb is converted to chloropyromorphite or Pb sorbed to Fe oxides [262]. Field treatment of high-Pb soil with soluble triple superphosphate or phosphoric acid caused a 69% reduction in soil Pb bioavailability to humans within 1.5 years [261]; rock phosphate is less reactive with soil Pb than the soluble phosphates although it may be cost-effective for acidic soils. Uptake of Zn, Cd, and Pb by normal grasses did not threaten wildlife (for example, [28]).

Because the cost of *in situ* phytostabilization of soil metals is so much lower than that of alternatives, this approach is receiving increasing consideration (for example, [260,263]). Unfortunately, for rice soils which induce human Cd disease, phytostabilization is not adequate to protect rice consumers; farmers would have to stop growing rice to avoid soil Cd risks. Phytoextraction of soil Cd, or change in crops grown is needed to protect consumers [65]. Because crop Zn protects against nearly all soil Cd risks, it is not evident that most cropland contaminated with geogenic 1:200 Cd:Zn ratio Zn contamination will require Cd remediation – rather, only Zn phytotoxicity remediation will be required and can be easily obtained with limestone and other soil amendments. Stuczynski *et al.* [264] fed plant cover from a remediated zinc smelter slag site in Poland compared with the same plant species grown on control soils without or with added Cd and Zn salts to equal the levels found in the plants from the contaminated site. Salt metals added to control hay caused much higher Cd accumulation in kidney and liver of calves than did the hay from the contaminated site. Plants growing on natural contaminated sites may be a valuable resource for selection of cover crops or metal-tolerant excluder species for revegetation of contaminated sites [31].

14.6 Recovery of Elements from Phytoextraction Biomass

Several authors have raised issues about disposal of phytoremediation biomass, suggesting that disposal will be so expensive that it will prevent many opportunities for commercial phytoextraction [14]. In the case of Ni, Au, Co, and perhaps Tl phytomining, biomass would be incinerated to produce energy, and the metal recovered from the ash would provide considerable profit potential [12,158,182].

The value of the biomass energy may be high enough to cover the cost of crop production, but not enough to make the technology profitable if the metals have no value. Radionuclides in biomass are clearly a more difficult issue because few incinerators are permitted to burn radioactive materials, and shipping the biomass to a permitted site could add substantially to the cost [265]. Biomass from Pb and As commercial phytoextraction has been disposed in normal landfills because the biomass was not hazardous according to US-EPA testing protocols [207].

Several groups have proposed biomass energy production (incineration or pyrolysis) with a side benefit of Cd phytoextraction using willow [266–271]. As noted elsewhere, high enough Cd in this ash would require its disposal in landfill rather than application to return nutrients to the forest where the biomass was produced. Although there has been considerable discussion of pyrolysis of biomass, there is no evidence that pyrolysis is ready to be deployed for phytoextraction biomass conversion. Further, because metals and nutrients are higher in leaves than wood, and ash from leaves can foul turbine generators which are direct-fired, phytoextraction biomass with leaves is an even greater issue. Disposal of the ash in landfill would incidentally increase the cost of growing the willow because more fertilizer and limestone would be needed if the ash were not returned to the field. It may be possible to use the high temperature of biomass incineration to separate volatile elements into the fly ash fraction and retain nutrients and alkalinity in cyclone and bottom ash [272] rather than be concerned about loss because of volatilization. Effective exhaust gas treatment systems are available to prevent metal loss during biomass burn.

Use of ash from Ni phytomining biomass as an alternative Ni ore has been demonstrated [12]. *Alyssum* ash was processed in an electric arc furnace and was a very successful Ni ore. Harris *et al.* [182] and Brooks *et al.* [158] also discussed the value of Ni phytomining biomass. Ni phytomining on serpentine soils rich in Ni, or on contaminated soils, can provide more profit than most common agronomic crops. Ni is much less volatile than Cd, Zn, Pb, and As [272] so it is relatively easy to retain Ni during biomass processing.

14.7 Risks to Wildlife during Phytoextraction Operations

High levels of Se and other metals in ingested plant phytoextraction biomass may be toxic to wildlife (for example, [146]). It is important to keep in mind that the sites being remediated have been identified because they cause metal risk to humans or the environment (wildlife). Lack of remediation continues the harm to wildlife. During remediation operations, sites will exclude large wildlife, but small mammals which are resident in small areas might be harmed. On the other hand, dig and haul will destroy all local wildlife [273].

Animal feeding tests with hyperaccumulator plants have not been reported, so no data are available to estimate the risk from ingestion of these plants. Another consideration is needed for plant Cd risk to animals. Most researchers cite NRC [147] as the authoritative source for tolerable levels of diet Cd. Readers should recognize that these limits are based on feeding diets with added metal salts, not crops with intrinsic Cd; Zn was not increased in proportion to Cd as occurs with most plant species. As noted above, increased Cd in test feeds with intrinsic Cd as high as $2.5 \text{ mg Cd kg}^{-1}$ caused no increase in kidney or liver Cd of test animals [274], so the $0.5 \text{ mg Cd kg}^{-1}$ suggested limit lacks a valid technical basis. Further, that limit was based not on animal health, but on protecting against increased Cd in liver and kidney used as human food. Such limits should be based on the bioavailable Cd taking into account the Zn present in the same plant material, not total Cd in the crop [65]. Stuczynski *et al.* [264] found that adding Cd salt to forage diets for calves gave considerably higher kidney and liver Cd than feeding forage grown on a phytostabilized Zn smelter slag site, and that omitting Zn addition caused greater Cd accumulation in tissues than when both Cd and Zn were added equivalent to the forage from the phytostabilized site.

In the case of *Alyssum* species used in Ni phytoextraction, the dense trichomes on all leaves strongly inhibit feeding by livestock and wildlife. In the Mediterranean serpentine soils where these species occur naturally, pastures rich in *Alyssum* with high Ni levels ($>1\%$ DW) are grazed by cows, sheep, goats and wildlife which avoid consuming *Alyssum*. In field tests of *Alyssum* species in Oregon, USA, cattle, deer and rabbits did not graze on *Alyssum* [12]. The trichomes give the plants a wiry texture which appears to be unpalatable to animals, thus protecting the animals from the high *Alyssum* Ni levels. The seeds contain $\sim 7000 \text{ mg Ni kg}^{-1}$ but are so small that they are not useful for forage/feed.

Selenium-accumulator plants can accumulate toxic levels of Se with little difficulty, and the Se is bioavailable and toxic to livestock and wildlife according to many veterinary toxicologists; Se accumulator species are considered to be toxic weeds [122] but these plants are seldom ingested by livestock due to the garlic odor of Se rich plants. Even crop plants without the selenate/sulfate selectivity of the Se hyperaccumulators may accumulate high enough of Se ($>3 \text{ mg kg}^{-1}$ DW) [123] that if the crop were 100% of livestock diets, the Se would be toxic based on NRC [147] estimates of levels of minerals tolerated by livestock. But as noted above, the Se-rich biomass could be used as a Se supplement for livestock feeds to replace the usual chemical Se addition.

14.8 Conclusions

The promise of phytoextraction, phytomining, phytovolatilization, and phytostabilization as important tools for society to deal with contaminated soils remains important despite the conclusions of others [282–285]. Phytoextraction goals should be based on reduction of risk, not on arbitrary soil metal concentrations that are not specifically related to risk. Food Cd risk is real on contaminated rice soils as discussed above, and reduction in soil Cd to allow production of rice with acceptable Cd levels may be achieved using high Cd-accumulating rice cultivars grown using nonflooded culture in acidic soil [109]. Yet, *T. caerulescens* did not perform adequately in rice soils because of the climate. But this

species is still promising for European and northern United States soils which comprise risk to humans. Risk levels to protect wildlife are estimated to be much lower than would be found if bioavailability were taken into account. And Cd does not magnify in terrestrial food chains [286].

Presumed risk from soil Zn and Ni based on additions of soluble metal salts to soils are woefully in error, as discussed in Chapter 17. When pH is adjusted to allow production of crops sensitive to excessive soluble soil Zn, lettuce can be grown on Zn smelter-contaminated soils and is safe for human consumption [287] even in soils with $100 \text{ mg Cd kg}^{-1}$ and $10\,000 \text{ mg Zn kg}^{-1}$. Nodulated white clover grows well in soils with high Zn if soil pH permits growth of the crop [288]. Thus, phytoextraction goals based on risk can be achieved by known hyperaccumulator crops. If the land involved has high value and taking it out of production in order to conduct phytoextraction would be unacceptable to land owners, they can use dig-and-haul methods with rapid remediation. For most arable soils, dig and haul will not be the rational choice for remediation.

Commercial phytoextraction practices continue to be developed and are being tested in the field. Because other soil remediation methods are so much more expensive, it seems likely that phytoextraction will continue to be developed. Improved crops will be bred for commercial application, and bioengineered plants will be developed with unique properties. Despite their technical value, bioengineered plants may not be accepted by the public even for phytoremediation [273,282], especially bioengineered strains of food plants.

Some phytoextraction can be profitable as a farming/phytomining business on contaminated or mineralized soils. Ni phytomining offers high profit potential [12]. Some ask why the technology has not been fully commercialized. The company (Viridian LLC) which licensed the patents obtained by Chaney, Angle, Li, and Baker [39,289] has chosen to not operate the technology by contracting with farmers to grow *Alyssum* crops on serpentine soils, but to attempt to do an Initial Public Offering of stock to recover their costs to support development of the technology and to obtain a profit. They have contracted with Vale-Inco to successfully test Ni phytomining on smelter-contaminated soils and mine waste deposits, and are considering phytomining on Vale-Inco properties. But this has not proceeded beyond planning, frustrating the scientists who considered the technology completely ready for commercial operations since 2001.

For most elements, phytoextraction can offer only lower cost of soil remediation. The biggest impediment to development and commercialization of many phytoextraction technology opportunities is the failure of environmental regulatory authorities to require remediation of highly contaminated soils. Until this market for soil metal phytoremediation service develops, only phytomining technologies will be practiced, along with basic research to understand hyperaccumulator plant biology and phytoextraction soil and plant chemistry.

Phytostabilization will remain a valid remediation technology for most contaminated sites. Mixed metal contamination can be handled by phytostabilization in most cases, except where food-chain transfer would continue risk to wildlife. This may be more important for soils with Zn and other metal phytotoxicity and with simultaneous Se or Mo contamination. Liming to alleviate Zn phytotoxicity would maximize Se or Mo accumulation in plants and threaten wildlife.

Public acceptance of *in situ* Pb inactivation will aid adoption of phytostabilization of mixed Zn-Pb-Cd contaminated sites such as the Joplin, MO, USA site [28,261] where field testing showed the forage was safe for livestock, and soil feeding tests showed strong

reduction in soil Pb bioavailability. Such remediated sites may not be permitted to become housing areas or playgrounds, in order to avoid exposure of children even to apparently remediated Pb risks. Controlled land use after phytostabilization can protect humans and the environment from soil trace element risks. Continued development of phytotechnologies will provide more choices for remediation, and demonstrate in the field the value to society these technologies offer.

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